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Research Summary

SUMMARY

Pharmacological approaches to the optimization of the oxygen affinity of liposome-encapsulated hemoglobin (LEH), a potential blood replacement fluid, have been studied. In our work we have researched the potential utility of LR16, L35, and related analogues in optimizing the oxygen affinity of LEH. Twenty drugs were synthesized and tested for their ability to modulate the P_{50} value of purified human hemoglobin. A new agent, 2-[4-(3,5-di(trifluoromethyl)-phenoxy]-2-methylpropionic acid (compound 02-50), was found to display activity greater than that of LR16, and approximately equivalent to the activity displayed by L35. At 02-50 concentrations of 0.75 mM and 1.5 mM, respectively, the P_{50} values of LEH preparations containing human hemoglobin stripped of 2,3-diphosphoglycerate were increased from 10 mm Hg to P_{50} values of 32 mm Hg and 61 mm Hg, respectively. Our data indicates that the allosteric modifiers LR16, L35, and 02-50 are capable of diffusing into LEH particles composed of distearoylphosphatidylcholine (DSPC): dimyristoylphosphatidylglycerol (DMPG): cholesterol [molecular ratios of 4:1:3, respectively] and decreasing P_{50} values, effectively.

Unfortunately, however, the desirable effects of the LR16, L35, and 02-50 agents on the encapsulated hemoglobin are seriously attenuated when serum albumin is present. Here we have shown that the ability of albumin to bind the allosteric modifiers of hemoglobin diminishes considerably their desired biological effects on hemoglobin. Whereas 1 mM LR16 shifts the P_{50} of hemoglobin free in solution from 8 mm Hg to a value of 49 mm Hg, physiologically relevant concentrations of 50 mg/ml human serum albumin right shift the oxygen dissociation profile of hemoglobin to control P_{50} values (8 mm Hg). In experiments with LEH, the addition of HSA to LEH suspensions containing LR16, L35, and 02-50 were found to seriously limit drug effectiveness. HSA is well known to have two binding sites for lipophilic and negatively-charged drugs, and the notion occurred to us that the allosteric modifiers of interest bind specifically with such a site. Experiments revealed, however, that the competition between the albumin versus hemoglobin binding was not attenuated by denaturation of HSA, indicating HSA-LR16 associations are nonspecific in nature.

In an attempt to overcome the diffusion of LR16 agents from the LEH particles, we synthesized a permanently-charged analogue of LR16. A permanent positive charge markedly diminishes the rate at which an agent can diffuse through a liposomal bilayer. The analogue which we synthesized, compounds 02-31 and 01-69 (iodide and BF_4 forms, respectively), was unfortunately found to display disappointingly low activity at modulating the oxygen affinity of hemoglobin. Because a viable pharmacological approach to the optimization of the P_{50} value of hemoglobin encapsulated within liposome remains a most attractive goal to pursue, alternate approaches other than the incorporation of quaternary ammonium salt moieties should be considered. Our experience with 02-50 indicates that LR16 can be substituted with larger, bulky

substituents actually resulting in the case of 02-50 in a marked increase in drug effectiveness at modulating LEH P_{50}. This data suggests further analogue development may allow for the incorporation of photoactive moieties into the drug structure, possibly allowing for covalent attachment of bioactive drug to the hemoglobin confined within the LEH particle.

EXPERIMENTAL METHODS

Drug Synthesis

2-[4-[[(aryl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid derivatives **26-37** were prepared by a two step reaction starting from commercial 4-aminophenol (Scheme 1). Its condensation in pyridine with isocyanates **2-13** led to intermediates **14-25**. To avoid a formation of by-products the isocyanates were added to the reaction mixture at 0°C and the reactions were carried out at this temperature for a period of about 15 min. Then, the reaction was continued at room temperature. In most cases high yields were obtained (over 90%). Urea derivatives **14-25**, while reacted with acetone-chloroform in the presence of NaOH followed by hydrolysis led to the sodium salts of the final products. The water suspension after reaction was washed with ethyl acetate to give solution of pure sodium salts. That modification omits a filtration, as it was described before - which is long and problematic. The final products were precipitated with 12% HCl.

Scheme 1

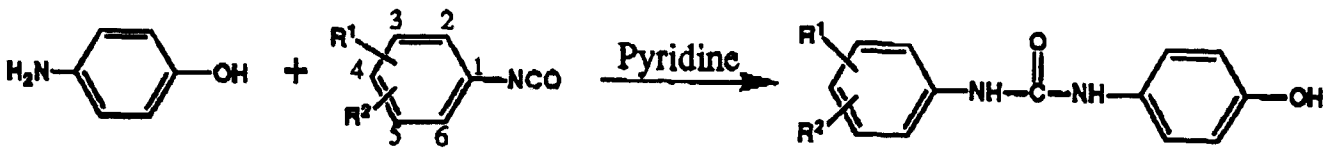
Attempts to prepare some thiourea analogues of known allosteric effectors according above reaction sequence failed. Condensation of corresponding 1-aryl-3-(4-hydroxyphenyl)-thiourea derivatives in acetone-chloroform-NaOH reaction led to a complicated mixtures of undefined products. Hence, thio analogues of **26** and **27** were finally prepared by a reaction of lithium salt of 2-(4-aminophenoxy)-2-methyl propionic acid (**41**) with 3-chloro- and 3,4-dichlorophenyl isothiocyanates (**38,39**) in pyridine with 58% and 52% yield, respectively (Scheme 2). This method was also successfully applied for the synthesis of 3,5-bis(trifluoromethyl) derivative **44** (44% yield), which could not be obtained previously according procedure shown on scheme 1, because of a partial hydrolysis of CF_3 groups in the reaction with acetone-chloroform, while an intermediate type **14-25** was refluxed in a strong alkaline conditions.

Schemes 2 and 3

Amino analogues of allosteric effectors of hemoglobin, possessing strong basic center on NH_2 group, can be easily accessible from a corresponding nitro derivatives by a catalytic hydrogenation (under 10 psi) on Pd/C in methanolic solution. Using this method compounds **45-49** were prepared with 44-90% yield. Till now, they were not reported in the literature and their effects on human hemoglobin were not investigated. Methyl ester of 2-[4-[[(3-aminophenyl)amino]carbonyl]amino]-phenoxy-2-methyl propionic acid (**50**), which was obtained as a by-product, while a crude amine (**46**) was filtered through silica gel in acidic methanolic solution, indicated much lower effect on hemoglobin as compared with compounds with free carboxylic group. The corresponding permethylated compound on amino group (**51**) was

synthesized (83% yield) according a similar procedure as it was described in the literature, using methyl iodide as an methylating agent. As an acceptor of evolved hydroiodide, diethyl aniline was used. In this case, both high purity of the starting amine and longer reaction times were required to obtain the salt in a pure, crystalline form.

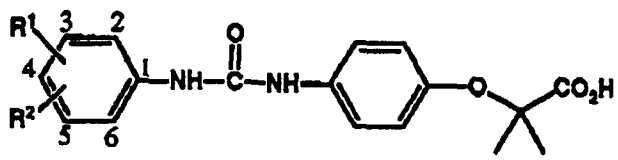
Scheme 1



1 2 - 13 14 - 25

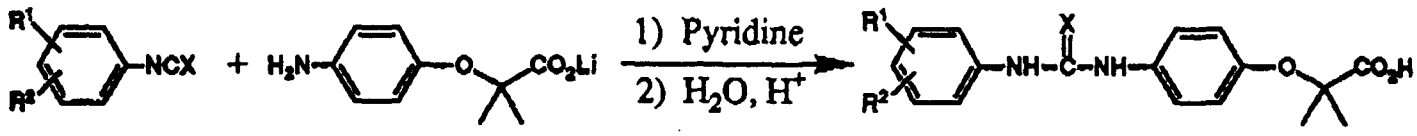
Compound	R^1	R^2
2,14,26	3-Cl	H
3,15,27	3-Cl	4-Cl
4,16,28	3-Cl	4-F
5,17,29	2-NO_2	H
6,18,30	3-NO_2	H
7,19,31	4-NO_2	H
8,20,32	2-OMe	4-NO_2
9,21,33	2-F	5-NO_2
10,22,34	2-Me	5-NO_2
11,23,35	3-NO_2	4-F
12,24,36	3-CN	H
13,25,37	4-CN	H

1) Me_2CO, $CHCl_3$
NaOH; reflux
2) H_2O, H^+



26 - 37

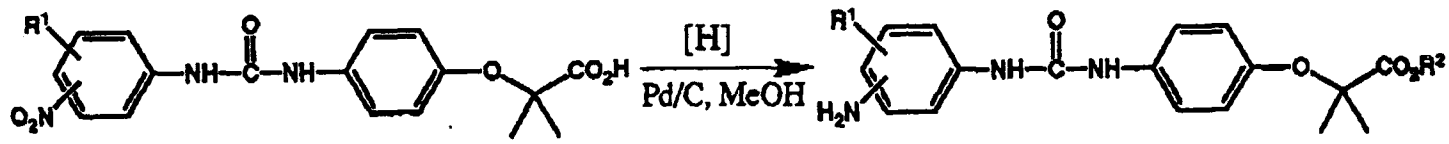
Scheme 2



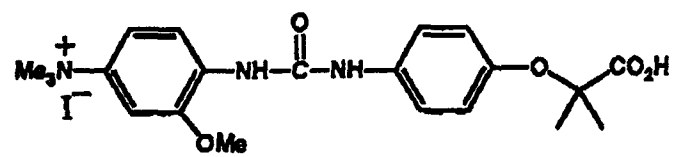
38 - 40 41 42 - 44

Compound	X	R^1	R^2
38,42	S	3-Cl	H
39,43	S	3-Cl	4-Cl
40,44	0	3-CF_3	5-CF_3

Scheme 3



29 - 32, 35 45 - 50



51

	45	46	47	48	49	50
-NH_2 position	2	3	4	4	3	3
R^1:	H	H	H	2-0Me	4-F	H
R^2:	H	H	H	H	H	Me

TLC was performed on precoated plastic sheets (0.2 mm) of silica gel 60 F-254 (E. Merck AG, Darmstadt, Germany); compounds were detected by UV lamps (254 nm). Hydrogenations were carried out in Parr Apparatus. Melting points were determined with a Buchi 530 apparatus and are uncorrected. NMR spectra were recorded for solution in DMSO-d_6 or $CDCl_3$/DMSO-d_6) (internal standard TMS) with a QE-300 (300 MHz) and Bruker AM-400 (400 MHz) spectrometers. Most of synthesized compounds has high tendency to associate water, hence for some of them elemental analysis was assessed for hydrates and subsequent high resolution-mass spectrometry confirmed empirical formula. Isocyanates and isothiocyanates were commercially available (Carbolabs - Bethany, Connecticut 06525; Aldrich - Milwaukee, Wisconsin 53201; Lancaster - Windham, New Hampshire 03087). 2-(4-Aminophenoxy)-2-methyl propionic acid (**41**) was obtained according a procedure described in the literature.[a,c]

Synthesis of 1-aryl-3-(4-hydroxyphenyl)urea derivatives (14-25). General Procedure.

Aryl isocyanate (**2-13**, 30 mmol) was added as a pure substance or as a solution in pyridine (1-2 mL) to a vigorously stirred solution of 4-aminophenol (3.3 g, 30 mmol) in pyridine (8-10 mL) at 0°C. The reaction was kept at this temperature for 15 min. Then, the ice bath was removed and the reaction continued for another 15 min at room temperature. Then, water (250 mL) was added, and the pyridine was neutralized with a small excess of 12% HCl (~ 180 mL). The suspension was left with stirring for 0.5 h, the precipitate was separated by filtration, washed with water, and dried to give products **14-25**. If the product was not pure enough (TLC:$CHCl_3$/MeOH - 10:1) it was dissolved in methanol and the insoluble white solid (products of polymerization of isocyanates) were filtered off. The filtrate was concentrated and dried *in vacuo*. Analytical samples were recrystallized (or chromatographed and recrystallized) from acetone, methanol or methanol/chloroform mixture.

Synthesis of 2-[4-[[(aryl)amino]carbonyl]amino]- phenoxy-2-methyl propionic acid derivatives (26-37). General Procedure.

A vigorously stirred mixture of sodium hydroxide (2.8 g, 71.6 mmol) and 1-aryl-3-(4-hydroxyphenyl)urea derivative (13.7 mmol) suspended in acetone (35 mL) was heated to reflux. Then chloroform (5.5 mL, 68 mmol) was added dropwise for about 10 min. The reaction was continued for an additional 4 h in reflux. Then, the solvents were evaporated and water (90 mL; for compounds **24,25** - 180 mL) to the residue was added. The mixture was shaken with ethyl acetate (2x40 mL), heated with charcoal at 50°C, filtered through Celite and then acidified with 12% HCl to pH ~ 1. The precipitate was isolated by filtration, washed with water and dried to give crude products **26-37**.

The products were purified according to the following procedure: The crude compound was dissolved in a small amount of acetone and then ethyl ether was added dropwise until a dark solid started to precipitate. The solution was left for 0.5 h and the solid was filtered off. The remaining solution was concentrated to dryness. This procedure was repeated 2-3 times. Finally, the product was precipitated from a concentrated acetone solution by addition of ethyl ether or crystallized from acetone, ether or acetone/ether mixture.

Synthesis of 2-[4-[[(aryl)amino]thiocarbonyl]amino]phenoxy-2-methylpropionic acid derivatives (42,43) and compound 44.

A solution of buthyllithium in hexane (1.6 N, 1.25 mL, 2 mmol) was added at the temperature 0°C to a solution of 2-(4-aminophenoxy)-2-methyl propionic acid (390 mg, 2 mmol) in pyridine (5 mL). The mixture was vigorously stirred and after 5 min an aryl isothiocyanate (**38,39**) or isocyanate **40** (2 mmol) was added. The reaction was carried out at 0°C for 15 min and then at room temperature for additional 15 min. The water (20 mL) was added and the pyridine was neutralized with a small excess of 12% HCl (~16 mL) to pH ~ 1. The oily precipitate was filtered, washed with water (4x10 mL), dissolved in acetone, filtered and concentrated to give crude compounds **42-44**. If necessary, they can be filtered through short column with silica gel ($CHCl_3$/MeOH as eluent) or purified by treatment with 10% NaOH (10 mL), washing with ethyl acetate (2x10 mL) and a precipitation by the addition of 10% HCl to pH ~ 1. Analytical samples were recrystallized from ether-acetone or acetone-chloroform mixture.

Catalytic Hydrogenation of compounds 29-32 and 35. General Procedure.

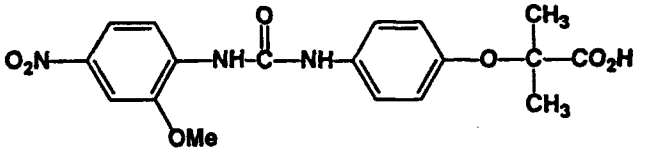
2-[4-[[(Nitroaryl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid derivative (2 mmole) was hydrogenated (10 psi) in MeOH (25 mL) using 10% Pd/C (80 mg) as a catalyst (10 psi) until the substrate disappeared (2-6 h; reaction was monitored on TLC, $CHCl_3$/MeOH - 4:1). After the reduction, the catalyst was filtered off through Celite, washed with MeOH, and evaporated to dryness (when the amine was partially soluble in MeOH, and evaporated to dryness (when the amine was partially soluble in MeOH, it was directly transformed in MeOH solution into corresponding hydrochloride). Crude product was suspended in water (15 mL) and it was acidified with HCl to pH ~ 1. The solution was washed with ethyl acetate (3x10 mL), the traces of ethyl acetate were removed from water phase under reduced pressure and the product was precipitated with ammonia (at pH ~ 6) or the solution was alkalized with NaOH and then it was acidified with acetic acid to give pure products (**45-49**).

2-[4-[[(2-Methoxy-4-trimethylamoniumphenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid iodide (51).

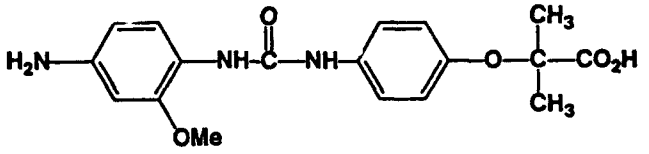
2-[4-[[(2-Methoxy-4-aminophenyl)amino]carbonyl]amino]phenoxy-2-methyl propionic acid (360 mg, 1 mmole) and diethylaniline (450 mg, 3.01 mmole) were dissolved in DMF (1.8 mL) and then methyl iodide (865 mg, 6.80 mmol) was added. The reaction was left with stirring at the room temperature for 4 days. It was filtered, the filtrate was concentrated and dried on the oil pump. The viscous oil was left until solidied. The chloroform (12 mL) was added and the precipitate was suspended using ultrasonic cleaners. Then, acetone was added (8 mL) and the white powder was isolated by filtration. The filtrate was concentrated and the procedure was repeated. 436 mg of the desired salt was obtained (83%); mp 186-190°C (acetone); ^{1}H NMR (400 MHz, DMSO-d_6) 9.29 (s, 1 H, NH), 8.44 (s, 1 H, NH), 8.27 (d, J = 9.6 Hz, 1H), 7.53 (d, J = 2.4 Hz, 1 H), 7.41 (dd, J = 9.6,2.4 Hz, 1 H), 7.32 (d, J = 8.6 Hz, 2 H), 6.80 (d, J = 8.6 Hz, 2 H), 3.99 (s, 3 H, OCH_3), 3.58 (s, 9 H, $N(CH_3)_3$), 1.45 (s, 6 H, 2xCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) 174.1 (CO_2H), 151.9, 150.0, 147.7, 140.3, 133.6, 130.3, 120.0 (2xC), 119.1 (2xC), 117.4, 111.7, 103.8, 78.6 (CCO_2H), 56.5 (OCH_3), 56.5 ($N(CH_3)_3$), 24.6 (2xCH_3). Anal. ($C_{21}H_{28}IN_3O_5$) C, H, N, I.

NEW LR16 ANALOGUES SYNTHESIZED AND EVALUATED

O1 - 36



O2 - 25



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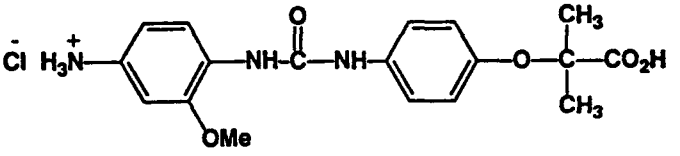

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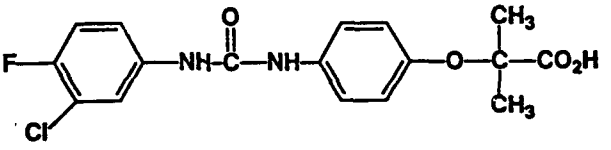

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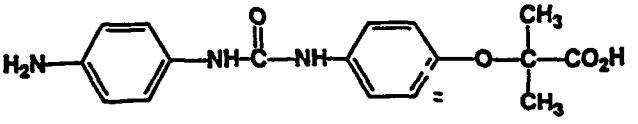

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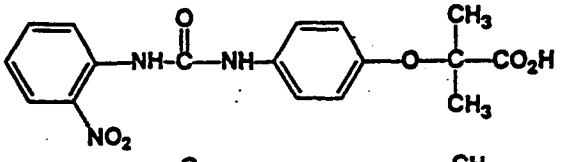

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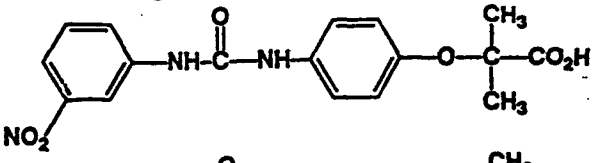

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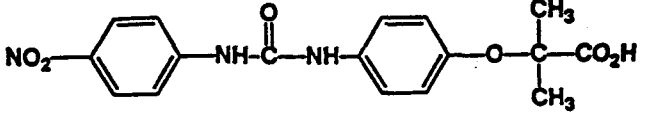

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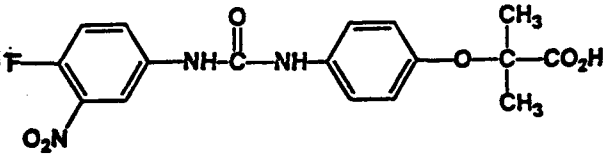

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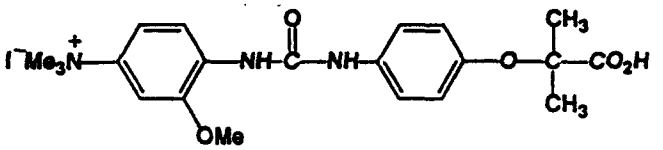

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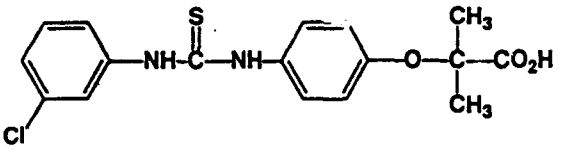

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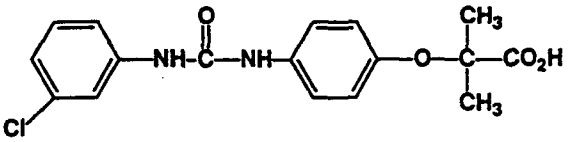

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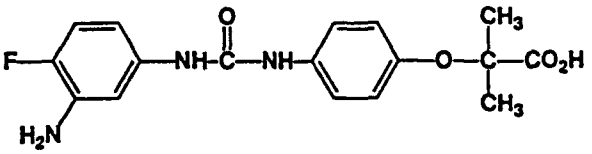

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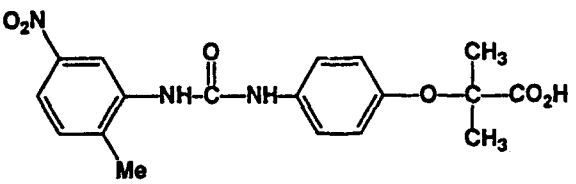

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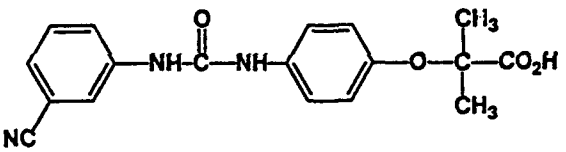

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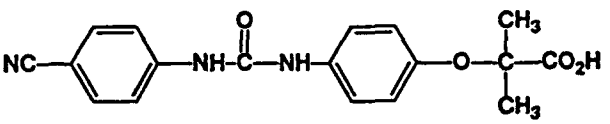

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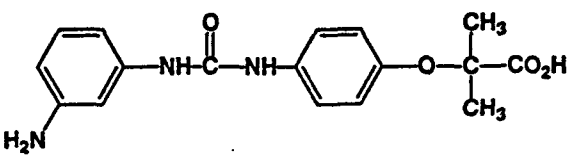

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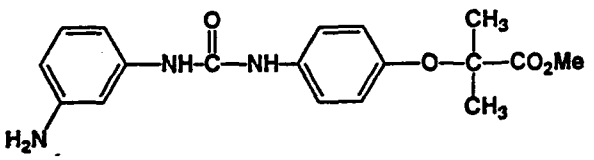
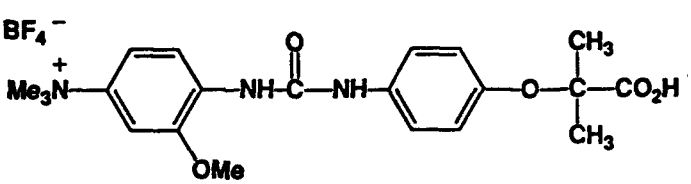
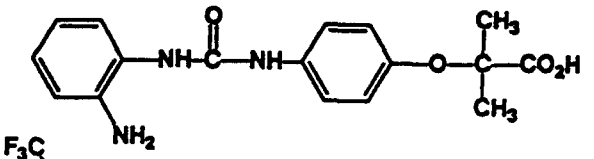


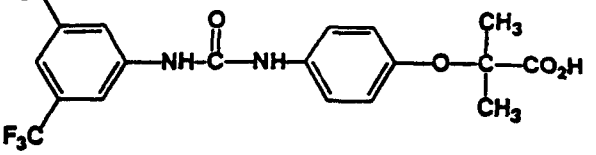
O1 - 69



O1 - 45



O2 - 50



Preparation of Purified Human Hemoglobin Solutions

Outdated blood, obtained from the Red Cross of Ohio, was centrifuged at 4°C at 1000 x g for 15-20 min. The supernatant was discarded, and the packed cells were carefully resuspended (*i.e.* without vigorous shaking) in an equal volume of cold 0.15 M NaCl and recentrifuged at 1000 x g. The 0.15 M NaCl washing was repeated four times.

The packed, washed cells in heavy glass centrifuge tubes were lysed by addition of an equal volume of cold purified water, followed by addition of chloroform (5% of the total volume). The mixture was stirred for 30 min at 4°C and then centrifuged at 3000 rpm for 10 min at 4°C to remove the major portion of cell debris and chloroform-containing viscous phase. The supernatant containing hemoglobin was centrifuged for 30 min at 4°C and 9000 x g. The supernatant containing hemoglobin was removed leaving behind the remaining cell debris.

Organic phosphates were removed by dialysing extensively at 4°C against 0.5 mM TES buffer containing 0.1 M NaCl, pH 7.5. The dialysis buffer was initially changed at one hour intervals for 6 hours followed by every 4-6 hours for next 24 hours. Finally, ionic impurities were removed by passing the hemoglobin solution through a column of Dowex MR-3 mixed bed ion exchange resin that was washed and pre-equilibrated with Millipore purified water at 4°C.

The purified hemoglobin solution was concentrated two-fold using Amicon Centriprep concentrators by centrifuging at 3000 x g and 4°C and stored at -20°C.

Drug Dissolution

Drug stock solutions of 3 mM or less in 2-{[tris-(hydroxymethyl)-methyl]-amino}-ethanesulfonic acid (TES) buffer (0.05 M TES, 0.14 M NaCl, pH 7.40) were prepared by vigorous vortexing. The mixture was sonicated briefly and/or warmed to 40°C if the dissolution process was slow as evidenced by a lack of optical clarity of the solutions. Finally, pH was adjusted to 7.40 if needed. LR16 stock solutions of >0.02 M were prepared by initially dissolving in TES buffer at pH 10-11 and then adjusting the pH to 7.40 with HCl.

P_{50} Determination

Recording of curves of equilibrium binding of oxygen to hemoglobin or LEH was carried out with the Hemox Analyzer (TCS Medical Products, Huntingdon Valley, PA). The operating principle of the Hemox Analyzer is based on dual-wavelength spectrophotometry for the measurement of the amounts of oxygenated and deoxygenated hemoglobin and a Clark membrane electrode for the measurement of the oxygen partial pressure.

Table I. Summary of the Effects of Allosteric Modifiers on Human Hemoglobin.[a]

Compound	P_{50}/P_{50C}
LR16	5.3
L35	6.3
01-22	1.7
01-35	1.3
01-36	1.4
01-37-1	0.9
01-37-2	1.6
01-38	2.2
01-40	1.8
01-42	2.0
01-43	2.1
01-45	1.5
01-69	1.0
02-24	1.3
02-25	1.2
02-26	3.1
02-30	3.1
02-31	0.9
02-38	1.4
02-39	2.0
02-40	1.9
02-50	6.2
02-51	1.6

[a] Drug and hemoglobin concentrations of 1.5 mM and 150 μM, respectively, were used. Experiments were conducted at 37 °C in TES buffer, pH 7.4. P_{50} values represent determinations in the presence of drug, while P_{50C} values represent determinations in the absence of drug. P_{50C} values ranged between 5 mm Hg and 10 mm Hg, depending on how efficiently the hemoglobin was stripped of its natural allosteric effector 2,3-DPG.

Briefly, an approximately 3 ml hemoglobin solution was drawn into the cuvette which also contained an oxygen sensitive membrane electrode, a thermistor probe for temperature measurement and a magnetic stirring bar. Measurements were carried out at 37°C. The sample was allowed to equilibrate with air flowing through the solution and the extent of oxygenation was recorded on X-Y recorder as a function of oxygen partial pressure. Figure 1 and 2 show typical association curves. The p_{50} values were calculated from oxygenation curves. A P_{50} value is defined as the pO_2 value at which 50% oxygen saturation of the sample occurs.

EVALUATION OF DRUG EFFECTIVENESS

Characterization of the Effectiveness of Allosteric Effectors at Modulating the Oxygen Affinity of Human Hemoglobin.

Table I summarizes the effect of the various allosteric modifiers of interest on the P_{50} value of purified hemoglobin stripped of its natural allosteric effector 2,3-DPG. Drug concentrations of 1.5 mM were employed. P_{50} refers to the partial oxygen pressure at which purified hemoglobin solution is half-saturated in the presence of 1.5 mM drug; P_{50C} refers to the partial oxygen pressure at which purified hemoglobin solution is half-saturated in the absence of drug. The P_{50C} values ranged from 5 mm Hg to 10 mm Hg; the observed variance existing due to differences in the level of purity of hemoglobin achieved for different preparations.

Of the new compounds tested, 02-50 was the most potent at modulating the oxygen binding pressure of hemoglobin. This analogue, when completely dissolved, was more effective than LR16 at modulating the P_{50} of hemoglobin free in solution. The 02-50 compound exhibited approximately the same level of effectiveness as L35 at modulating the oxygen binding properties of hemoglobin. In an attempt to overcome the diffusion of LR16 agents from the LEH particles, we synthesized a permanently-charged analogue of LR16. A permanent positive charge markedly diminishes the rate at which an agent can diffuse through a liposomal bilayer. The analogue which we synthesized, compounds 02-31 and 01-69 (iodide and BF_4 forms, respectively), was unfortunately found to display disappointingly low activity at modulating the oxygen affinity of hemoglobin.

Reductions in Drug Effectiveness Due to the Presence of Human Serum Albumin.

Figure 1 shows how the addition of human serum albumin modulates the effect which 2 mM LR16 exerts on hemoglobin free in solution at a concentration of 150 μM. In the absence of HSA LR16 has a strong effect on hemoglobin, shifting the curve well to the right with a P_{50} value of 44 mm Hg. Upon incremental HSA addition, however, the P_{50} shifts back to close to control values for purified hemoglobin in the absence of drug. The addition of HSA at concentrations of 10 mg/ml, 15 mg/ml, and physiologically relevant 40 mg/ml resulted in P_{50} values of 33 mm Hg, 24 mm Hg, and 8 mm Hg, respectively. Control values in the absence of drug were determined to be 6 mm Hg. Figure 2 demonstrates the ability of HSA to attenuate the effect of our new 02-50 analogue in LEH preparations. LEH plus 0.75 mM drug yielded a P_{50} value of 32 mm Hg. The addition of HSA concentrations of 5 mg/ml and 15 mg/ml to the LEH-drug suspension reduced the observed P_{50} values to 13 mm Hg and 11 mm Hg, respectively. The control value for LEH only (*i.e.* no drug or HSA) was 10 mm Hg.

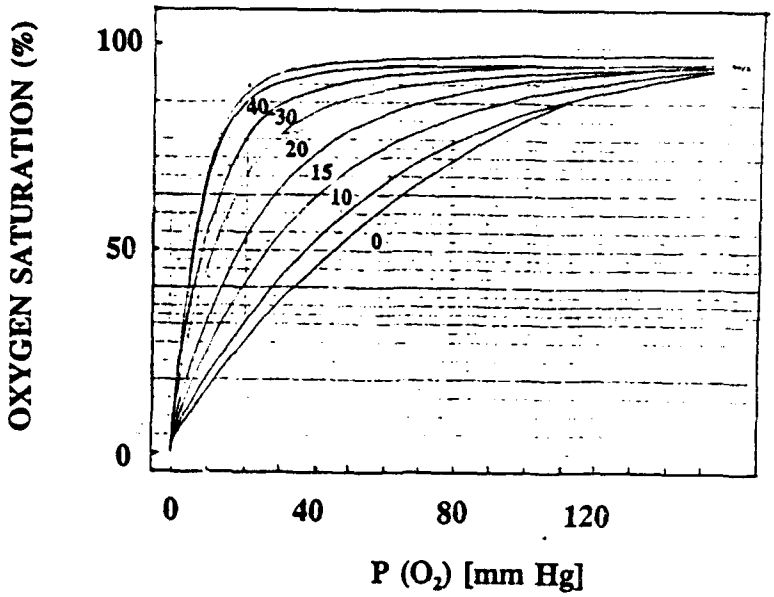


Figure 1. Oxygen dissociation curves showing how varying amounts of HSA modulate the effects of 2 mm LR16 on purified human hemoglobin (150 μM). HSA concentrations of 0 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml, 30 mg/ml, and 40 mg/ml were studied. The left most curve represents hemoglobin solutions in the absence of LR16.

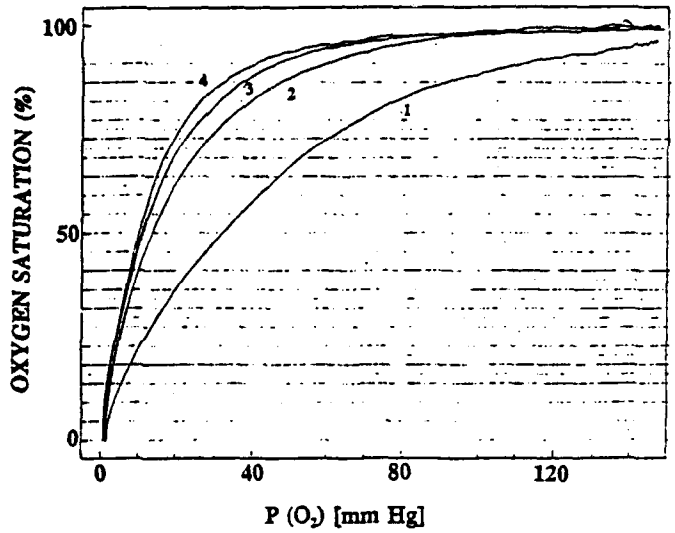


Figure 2. Oxygen dissociation curves showing how varying amounts of HSA modulates the effect of the new 02-50 agent on the oxygen affinity of LEH. Curve 1 shows the profile for LEH with 0.75 mM 02-50 present. Curve 2 shows the effect of addition of 5 mg/ml of HSA, while curve 3 shows a further decrease in P_{50} value upon increasing HSA concentration to 15 mg/ml. The left most curve represents the oxygen dissociation profiles for LEH suspensions in the absence of 02-50. Our data indicates that the allosteric modifier 02-50 diffuses out of LEH particles when HSA is present. LEH particles contained distearoylphosphatidylcholine (DSPC): dimyristoylphosphatidylglycerol (DMPG): cholesterol [molecular ratios of 4:1:3, respectively].

Abstracts/Manuscripts

Burke, T.G., Asmerom, Y., Singh, A. and Rahbar, S. Liposome-encapsulated Hemoglobin: Use of LR16 Analogues in the Optimization of Its Oxygen Binding Properties. Biophys. J., 59: 175a (1991).

Burke, T.G., Ostrowski, S., Rahbar, S., and Priebe, W. Liposome-Encapsulated Hemoglobin as a Blood Substitute: Synthesis and Evaluation of Allosteric Effectors for the Optimization of Oxygen Affinity, Pharmaceut. Res. 9: S-74 (1992).

Ostrowski, S., Burke, T.G., and Priebe, W. ^{13}C NMR Spectra of Allosteric Effectors of Hemoglobin, Journal of Magnetic Resonance, in review.

Burke, T.G., Rudolph, A.S., Mishra, A.K., Ostrowski, S., and Priebe, W. Liposome-Encapsulated Hemoglobin as a Blood Substitute: Evaluation of Allosteric Modifiers For the Adjustment of Oxygen Affinity, manuscript in preparation.

Burke, T.G., Ostrowski, S., Mishra, A.K., and Priebe, W. New Methylpropionic Acid-Derived Modifiers of Human Hemoglobin Hemoglobin, manuscript in preparation.